

Amendments to the Specification

Please replace paragraph [0001] with the following amended paragraph:

[0001] This application is a divisional application of U.S. Serial No. 09/692,325 filed October 20, 2003, now U.S. Patent No. 6,632,435, which claims the benefit of U.S. provisional application Serial No. 60/160,633 filed October 20, 1999 and U.S. provisional application Serial No. 60/187,871 filed March 8, 2000.

Please replace paragraph [00058] with the following amended paragraph:

[00058] The alanine-scan acid termini peptides (Table I) were synthesized on a ~~Pioneer~~ PioneerTM Peptide Synthesizer with MPS Accessory (~~Perseptive Biosystems~~ BiosystemsTM, Foster City, CA) at 0.05 mmol scale using Fmoc-L-Val PEG-PS resin and standard L-Fmoc amino acids with HATU/DIPEA activators. The peptide 44/46 amidated sublibrary (Table IV) was synthesized using standard Fmoc protocols, using a ~~Synergy~~ SynergyTM (~~Perseptive Biosystems~~ BiosystemsTM 432A, Foster City, CA) at 0.025 mmole scale. Amidated peptides were synthesized using the Fmoc protected Val-Rink Amide-MBHA resin (~~AnaSpec~~ AnaSpecTM Inc., San Jose, CA). The peptide 44/46 free acid sub-library (Table IV) also was synthesized as described above using Fmoc Val ~~Wang~~ WangTM Resin (~~AnaSpec~~ AnaSpecTM Inc., San Jose, CA). The Fmoc L-amino acids used in all syntheses were purchased from ~~Novabiochem~~ NovabiochemTM (San Diego, CA) and Perseptive ~~Biosystems~~

BiosystemsTM (Foster City, CA). Fmoc-tyrosine, serine, and threonine had t-butyl side chain protection and asparagine had trityl side chain protection.

Please replace paragraph [00059] with the following amended paragraph:

[00059] All peptides were cleaved from the resin and purified according to prior art methods (39). To verify correct mass of the peptide, Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI) was performed on the ~~Kompact~~ KompactTM Probe (Kratos ~~Analytical~~ AnalyticalTM, Manchester, UK). HPLC (~~Schimadzu~~ SchimadzuTM SCL 10AVP) using a C₁₈ column of 4.6x250 mm dimension composed of 5µx300Å Particles (Vydac ~~Separations~~ SeparationsTM, Hesperia, CA) also was performed. Sample purity was assessed at 70-80% based on the area percent of the sample peak in the chromatogram. The native pp65495-503 epitope was purchased from Peninsula ~~Laboratories~~ LaboratoriesTM Inc. (San Carlos, CA) at a purity of 95% established by HPLC.

Please replace paragraph [00060] with the following amended paragraph:

[00060] Peptide loaded antigen presenting cells were prepared as follows. Sixty milliliters of human blood was collected in sodium heparin from healthy donors who were serologically typed as HLA A*0201 and HCMV positive. Donors were first screened for HLA type (COH HLA Typing laboratory or National Marrow Donor program) and were subsequently test for HCMV seropositivity by indirect immunofluorescence (Hemagen ~~Diagnostic~~ DiagnosticTM,

Columbia, MD). Peripheral blood mononuclear cells were separated using Ficoll-Paque™ (~~Amersham Pharmacia~~ Pharmacia™, Uppsala, Sweden), and cells were resuspended in phosphate-buffered saline (PBS) and washed two times.

Please replace paragraph [00061] with the following amended paragraph:

[00061] Sixteen to twenty-four million of the ficoll-separated peripheral blood mononuclear cells were incubated 2-3 hours at 37°C in a 5% CO₂ incubator with 100 µM HCMV pp65₄₉₅₋₅₀₃ peptide (SEQ ID NO: 1) in a volume of 100 µl of T cell culture medium (IVSTCM). IVSTCM consists of RPMI-1640 supplemented with 20% heat inactivated HAB serum, 10 IU/ml r-IL2 (~~Chiron~~ Chiron™, Emeryville, CA); 25 mM Hepes Buffer Solution, 50 U/ml penicillin, 50 µg/ml streptomycin, 2 mM L-glutamine and 0.5 mM sodium pyruvate (~~Gibco~~ Gibco™, Rockville, MD). Human AB⁺ and HCMV seronegative serum (HAB) for IVS studies was obtained from plasma screened for blood group and HCMV status. Extraction of serum from plasma was performed following standard techniques. Pooled HAB serum was heat inactivated at 56°C for 30 minutes, tested for its ability to support T cell growth, and stored at -20°C. After the incubation period, the cells, now presenting pp65₄₉₅₋₅₀₃ were resuspended in 2 ml IVSTCM, and γ-irradiated (2,400 rads) using the Isomedic Model 19 Gammator (Nuclear Canada, Parsippany, NJ).

Please replace paragraph [00062] with the following amended paragraph:

[00062] During the peptide incorporation procedure, an additional aliquot of 10-20 million cells from the same freshly separated peripheral blood mononuclear cells was depleted of CD4⁺, CD16⁺, and CD56⁺ cells as follows. The cells were incubated with purified mouse anti-human CD4, CD16 and CD56 monoclonal antibodies (mAb) (~~Pharmingen~~ PharmingenTM, San Diego, CA) at 10 fold of their saturating concentration, for one hour, with gentle mixing at 4°C. M450 ~~Dynabead~~ DynabeadTM Dynabead goat anti-mouse IgG (~~Dynal~~ DynalTM, Oslo, Norway) were then added to the mAb labeled PBMC to effect indirect immunomagnetic separation. The resulting population was >80% CD8⁺ as determined using a FACSCalburTM fluorescence activated cell sorter (BD Immunocytometry ~~Systems~~ SystemsTM, Palo Alto, CA). These depleted PBMC effectors (200,000 ~~cells/ml~~ cells/ml) were mixed with an equal amount of antigen presenting cells (5 million cells/ml), and plated in a 24-well plate at 2 ml/well. The CTL effectors were incubated at 37°C, in a 5% CO₂ incubator for two weeks. They were fed with 10 IU/ml rIL-2 on days 5 and 10, and fresh medium when necessary.

Please replace paragraph [00063] with the following amended paragraph:

[00063] HCMV specific T cell clones were obtained by limiting dilution from the stimulated CTL generated in Example 2. Frozen or fresh cells from the cultures obtained in Example 2 were plated in 96-well U-bottom plates on day 15 of culture at a concentration of 1 or 3 cells/well together with 150,000 γ-irradiated fresh allogeneic peripheral blood mononuclear cells

and 0.5 µg/ml phytohemagglutinin (~~Murex~~ MurexTM, Dartford, UK) in a final volume of 150 µl of T cell cloning medium (20). After 14 days, proliferating cells from single wells were transferred into single wells of 24-well plates and restimulated a second time with 1 million/well/ml fresh irradiated allogeneic PBMC and PHA as described above. Starting from day 8 after the second restimulation, actively expanding clones (5 to 30 million cells) were tested in a chromium release assay for the ability to specifically lyse cells.

Please replace paragraph [00064] with the following amended paragraph:

[00064] Chromium release assays were performed according to the methods of (20). Briefly, target cell lines (antigen presenting cells which present pp65₄₉₅₋₅₀₃) were labeled for one hour with ~~200 µCi/ml ⁵¹Cr (ICN~~ 200 µCi/ml ⁵¹Cr (ICNTM, Costa Mesa, CA). HCMV infected targets were prepared as follows. Five-hundred thousand fibroblasts were pretreated with 800 U/10⁶ recombinant IFN-γ, (~~Preprotech~~ PreprotechTM, Rocky Hill, NJ) to upregulate MHC class I expression and HCMV was added at an m.o.i. of 4. Cells were incubated with virus for two hours at 37°C, in a 5% CO₂ incubator. Fresh FBM containing IFN-γ was then added and the cells incubated for an additional 14 hours. Infected fibroblasts were trypsinized, labeled with ⁵¹Cr as described above and used in the assay at an effector-to-target ratio (E/T) of 50 and 10. Epstein-Barr virus transformed B cell lines (EBVLCL) were generated following standard technology using the supernatant of EBV-infected marmoset cells. EBVLCL were grown in medium

consisting of RPMI-1640 (~~Gibco~~ GibcoTM, Rockville, MD) supplemented with 10% FBS, 10 mM Hepes Buffer Solution (Irvine ~~Scientific~~ ScientificTM, Santa Ana, CA), penicillin, streptomycin and L-glutamine as described for fibroblast medium. The cells used as targets were prepared by pulsing with 50 μ m pp65₄₉₅₋₅₀₃ peptide, and used at an E/T of 25 and 5. For PS-SCL screening and titration of analog peptides, T2 cells, maintained as described in Diamond et al., Blood 90:1751-1767 (1997), were pulsed with serial peptide dilutions for use as targets for T cell clone effectors at an E/T of 5.

Please replace paragraph [00065] with the following amended paragraph:

[00065] In the assay, loaded targets and effectors were mixed in culture and incubated for four hours, at which time chromium release was measured. Supernatants were collected on filter frames (~~SKATRON~~ SkatronTM, Oslo, Norway) and radioactivity determined in a Cobra II gamma counter (~~Packard~~ PackardTM, Meriden, CT). For each assay, spontaneous release from the target cells in the absence of effector CTL and maximum possible release after treatment with 2% SDS (Baker ~~Chemicals~~ ChemicalsTM, Phillipsburg, NJ) was determined. Specific cytotoxicity was defined as: $100X [(Re-Rs) / (Max - Rs)]$, where Re= experimental release, and Rs= spontaneous release. Chromium release assays in which lysis without addition of peptide was 20% or greater, or in which spontaneous lysis was 30% or more of the maximal release were considered unacceptable and not reported.

Please replace paragraph [00080] with the following amended paragraph:

[00080] HLA A2-Ig dimer aliquots were individually loaded with a series of peptides, and incubated with two different T cell clones, 3-3F4 and VB57. The soluble HLA-A2-Ig complexes (HLA dimers) were loaded with peptide according to the methods of Greten et al., Proc. Natl. Acad. Sci. USA 95:7568-7573 (1998). For each peptide analog to be tested, 10 µg of HLA dimers in 10 µl PBS containing 0.02% NaN₃ (Baker ~~Chemical~~ ChemicalTM, Phillipsburg, NJ) was incubated for three days at 4°C with 660-fold molar excess peptide. Dimers also were loaded with the pp65₄₉₅₋₅₀₃ native peptide, an irrelevant peptide (pp54 B*0702 epitope), and 2 µl PBS buffer containing 0.02% NaN₃, in all experiments to serve as controls. One microgram aliquots of peptide loaded dimers were mixed with 0.2 million of either T cell clone 3-3F4 or VB57 for 75 minutes on ice. Cells were washed in PBS containing 2% FBS and 0.02% NaN₃ (WB), and subsequently stained with 0.9 µl/sample of goat anti-mouse IgG₁-PE (~~Caltag~~ CaltagTM, Burlingame, CA). After two additional washings in WB, cells were analyzed by fluorescence-activated cell sorting (FACSCaliburTM). Specific fluorescence was calculated as fluorescence ratio between the mean fluorescence of the peptide-loaded dimers and the mean fluorescence of the buffer-loaded control dimers. See Figure 4. Peptides names ending in N are amidated, while those ending in C are free acids. Each peptide-loaded dimer complex was tested at least twice with two different T cell clones (3-3F4 and VB57). The fluorescence indices indicated in the Figure are an average of both

experiments. Measurements with both cell lines were normalized, so they could be displayed on the same axis.

Please replace paragraph [00082] with the following amended paragraph:

[00082] Ten randomly selected HCMV-seropositive, HLA A*0201⁺ healthy donors were selected for evaluation to confirm that the pp65₄₉₅₋₅₀₃ CTL epitope is widely recognized by HLA A*0201 persons. The haplotypes of examined individuals are shown in Table III. A one-step *in vitro* stimulation procedure modified from Lalvani et al., J. Immunol. Meth. 210:65-77 (1997), was carried out utilizing the pp65₄₉₅₋₅₀₃ CTL epitope (SEQ ID NO:1) as the immunogen. See LaRosa et al., Blood 92(10, Suppl. 1):518a (1998). In every case, the HLA A*0201 donors provoked a specific CTL response against T2 cells, and against HCMV-infected fibroblasts. See Figure 5B. AD169 strain HCMV was provided by J. Zaia (City of Hope Medical Center, COH). Virus stocks of 5-10 x 10⁶ pfu/ml were prepared from the supernatant of infected MRCvhhm5 fibroblasts as previously described in Diamond et al., Blood 90:1751-1767 (1997) and used to infect dermal fibroblasts. Adherent cell lines were grown in fibroblast medium (FBM) consisting of D-MEM (~~Gibco~~ GibcoTM, Rockville, MD) supplemented with 10% FBS (~~Hyclone~~ HycloneTM, Logan, UT), 50 U/ml penicillin, 50 µg/ml streptomycin (~~Gibco~~ GibcoTM, Rockville, MD) and 2 mM L-glutamine (~~Gibco~~ GibcoTM, Rockville, MD). The results demonstrate that HCMV infection in HLA A*0201 positive healthy donors stimulates a specific immune response to SEQ ID NO:1 that

is independent of haplotype, and likely to be universal in its expression.

Please replace paragraph [00092] with the following amended paragraph:

[00092] T2 cells, which express HLA-A*0201 molecules, were cultured in IMDM (~~Gibco~~ GibcoTM, Rockville, MD) with supplements as described for EBVLCL. For each analog to be tested, 250,000 T2 cells were washed twice and resuspended in serum free IMDM and incubated overnight with 100 μ M peptide together with 15 μ g/ml human β_2 -microglobulin (~~Sigma~~ SigmaTM, Lt. Louis, MO) at 37°C in a 5% CO₂ incubator. Peptide loaded cells were washed once at 4°C with cold PBS containing 0.05% BSA (~~Sigma~~ SigmaTM, St.; Louis, MO) and 0.02% NaN₃ followed by incubation with 1 μ g/sample murine mAb BB7.2, an antibody specific for cell-surface HLA A*0201 (ATCC), for 30 minutes at 4°C. After washing twice with cold buffer, FITC-conjugated rat anti-mouse IgG_{2a/2b} (1:40 dilution; ~~Pharmlngen~~ PharmlngenTM, San Diego, CA) was added and incubated for another 30 minutes at 4°C. The cells were washed twice in cold buffer, and mean fluorescence intensity (MFI) of 10⁴ gated cells was measured using a FACSCaliburTM.